

EXPERIMENTAL TRYPANOSOMA BRUCEI INFECTION
AND IMMUNITY IN VARIOUS SPECIES OF
PEROMYSCUS (AMERICAN DEER MICE).

By

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Introduction.

The American rodent genus *Peromyscus* (popularly known as wild mice, deer mice, vesper mice, white-footed mice, etc.) has never been investigated as to its susceptibility to infection with *Trypanosoma brucei* (5, 18). Since various species and subspecies of this genus are very widely distributed in the United States, Canada, and Mexico and are restricted in nature to the North American continent (11), such an inquiry was considered desirable.

A preliminary series of experiments was undertaken during 1931 which revealed very striking and clear-cut differences in susceptibility between various species of *Peromyscus*. These preliminary findings aroused the writer's interest to undertake a systematic study. As further experiments were performed on several species, subspecies and hybrids belonging to this genus, further interesting results were obtained. The present report is by no means exhaustive, because there are many species and subspecies found in nature which are not included in this investigation (11). Furthermore, certain experiments are still in progress. However, the data on hand are sufficiently conclusive to prompt the writer to make a brief report.

Materials and methods.

Experimental animals. Various subspecies of *Peromyscus* were supplied by Dr. Lee R. Dice, Curator of Mammals of the University of Michigan. Dr. W. H. Feldman, Director of Vertebrate Genetics, University of Michigan, generously supplied several pure strains, hybrids and albino and hairless mutants of *maniculatus*, also *Rattus*

* From the Hygienic Laboratory, University of Michigan, Ann Arbor, Michigan.

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rattus, *Mus bactrianus*, and hairless *Mus musculus*. The writer is greatly indebted to these two investigators for their kind cooperation.

Individuals of various species of *Peromyscus*, which had been captured in a wild state, were examined for possible natural infection with trypanosomes (17). The usual examination consisted of direct microscopical examination of blood (obtained from the tail). In a few instances a sample of heart blood was placed in culture media known to support the growth of trypanosomes (7). The writer was unable to detect any natural infection in these deer mice.*

The strain of Trypanosoma brucei. Our nagana material was derived from that which was sent to England by Bruce in 1896 by Dr. Waghorn. It is the same as that used by English and French investigators. This Zululand parasite was sent from England, by the courtesy of Miss Florence Durhams and Dr. W. Mitchell, to McGill University. Dr. H. Wolferstan Thomas, then a Fellow of the McGill Medical School, supplied Professor Novy with nagana blood (1903). Since then Professor Novy has kept this strain in guinea pigs at the University of Michigan. At the time of the experiments, this strain killed guinea pigs in from 16 to 22 days, while *Mus musculus*, *Mus bactrianus*, and *Rattus norvegicus* invariably died within a week after inoculation with nagana blood derived from guinea pigs or rabbits.

Initial inoculation. In all the *Peromyscus* used, experimental nagana was produced by intraperitoneal inoculation with the same strain of *Trypanosoma brucei*.

A rat, guinea pig, or rabbit at the height of infection with nagana, when the trypanosomes were swarming in the circulation, was bled, the rats and guinea pigs by heart puncture and the rabbits from the ear. The blood was at once defibrinated and diluted with an equal amount of physiological salt solution (0.9 percent NaCl) or with an equal volume of Tyrode solution. Suspensions prepared in this way contained large numbers of active trypanosomes. One loop of such material, when examined with a no. 6 objective, showed about fifty trypanosomes per microscopic field.

The deer mice were all inoculated with the same dose of infective nagana blood, namely, 0.25 cc. of heavy suspension, within a very short time, usually averaging about one minute for each mouse. Thus over fifty mice were inoculated within an hour. After the last inoculation of *Peromyscus* groups, a number of colored, white and hairless mice

* This paper was already finished when the writer found that newly captured *Peromyscus* were infected with their own trypanosome. Further work on this trypanosome is in progress.

(*Mus musculus*), rats, guinea pigs and occasionally a rabbit, were inoculated as controls for determination of virulence and as a check on the inoculum. Whenever less than three *Peromyscus* were inoculated at a given time, the inoculations were performed either with two drops of ear blood from a nagana guinea pig, or with tail blood from a nagana rat at the height of infection. The blood was taken up into a syringe containing 0.5 cc. salt solution and was inoculated into the animals.

Reinoculations. A few mice (*P. l. noveboracensis*) were reinoculated on several occasions with *Trypanosoma brucei*. Such inoculations were made with from 0.25 to 0.5 cc. of inoculum containing about fifty percent of nagana blood thickly populated with trypanosomes.

Microscopical examination for the determination of infectivity, crises and relapses. The tail of the mouse was seized gently with long forceps and its tip was pushed through the screen on the top of the glass jar. With the aid of another pair of forceps the root of the tail was held from the outside, thus suspending the mouse in the jar. With sterilized scissors, a very small portion of the tip of the tail was cut and a cover glass held by Novy forceps was touched to the tail blood. A small droplet of blood adhered to the cover glass which was placed on a slide. The cover slip was pressed gently with the forceps to give a uniform thickness to the preparation, which consisted of one to two layers of blood cells.

With sufficient experience, one can identify and estimate the average number within a minute, using a no. 6 objective. When trypanosomes were numerous, a shorter observation was found sufficient to warrant a recording. However, when they were scanty or "none found," five to thirty minutes or more were spent in searching for a single trypanosome. Such examinations were performed at frequent intervals and the results recorded.

Susceptible test animal inoculation. This consisted in the inoculation of two to three drops of tail blood from a given *Peromyscus* into *Mus musculus*, *Rattus norvegicus* or both. Occasionally a guinea pig or rabbit was also inoculated for the same purpose. The rats and mice were inoculated intraperitoneally, rabbits and guinea pigs subcutaneously.

The infection with *Trypanosoma brucei* and the time of death of these animals was recorded. Whenever test animals failed to contract the disease (this usually happened only with inoculum derived from *P. l. noveboracensis* several months after its initial inoculation) they were kept under observation from three to six months before they were discarded as negative.

Experimental findings.

The results of this study are summarized in table 1, and discussed in the latter part of this paper. Various tables dealing with records of crises and relapses of trypanosomes, test animal inoculations, and the histories of experimental animals have been omitted because of lack of space (11).

Species in which nagana infection ran an acute course.—Individuals of *P. c. californicus*, *P. c. insignis*, *P. e. eremicus*, *P. e. anthonyi*, and *P. p. polionotus* all contracted the disease readily. After a short incubation period, trypanosomes appeared in the circulation; the number of parasites increased progressively and rapidly until the blood of the animals was swarming with them. No trypanolytic crises were demonstrable. Their blood was fully virulent to the usual test animals. Not a single individual of these forms survived longer than 16 days. The minimum period of the disease was four days, while the general average was from five to seven days. (See table 1.)

The mice did not appear ill until about six to twelve hours before death. At this period they sat quite still and were practically insensitive to external stimuli. Whenever chemical blood examinations were performed, during the latter part of the disease, they showed a distinct hypoglycemia. At autopsy the most constant and marked gross pathological feature was extreme enlargement of the spleen.

Species in which nagana infection followed a subacute course.—The following subspecies of *maniculatus* were inoculated with *Trypanosoma brucei*: *P. m. artemisiae*, *P. m. bairdii*, *P. m. blandus*, *P. m. gambelii*, *P. m. osgoodii*, *P. m. rubidus*, *P. m. rufinus*, and *P. m. sonorensis*. In these animals the disease ran a subacute course, frequent crises and relapses being noted. During the life of the animals, they harbored *Trypanosoma brucei* in the circulation, the number of parasites varying somewhat among the different subspecies and in the same individual at different stages of the disease. Some individuals developed blindness during the latter part of the infection. A few of these mice lived as long as 224 days, the minimum period being 23 days, and the average duration of the disease over 80 days.

From the first sign of infection until the death of the animal, the blood was infective and fatal to susceptible laboratory animals (i.e., *Rattus rattus*, *Rattus norvegicus*, and *Mus musculus*), killing them within 5-15 days, depending upon the number of trypanosomes inoculated and upon the source of the inoculum.

Albino and hairless *P. m. gambelii* likewise contracted subacute infections, the average duration of the disease being over 50 days, while

TABLE 1.

*Experimental nagana in various species of mammals.**

Species	No. animals inoculated with <i>Tr. brucei</i>	Fatality in days			Remarks	
		Min.	Max.	Aver.	Crises and relapses of trypanosomes	Type of infection
<i>P. c. californicus</i> . . .	4	5	6	5.5	No crisis	Acute, always fatal
<i>P. c. insignis</i>	2	7	7	7.0	No crisis	Acute, always fatal
<i>P. e. anthonyi</i>	3	4	14	8.3	No crisis	Acute, always fatal
<i>P. e. eremicus</i>	17	6	10	7.5	No crisis	Acute, always fatal
<i>P. p. polionotus</i>	9	4	10	6.3	No crisis	Acute, always fatal
<i>P. m. artemisiae</i>	12	31	220	74.1	Frequent crises and relapses	Subacute, always fatal
<i>P. m. bairdii</i>	13	21	179	73.1	Frequent crises and relapses	Subacute, always fatal
<i>P. m. blandus</i>	16	23	101	60.6	Frequent crises and relapses	Subacute, always fatal
<i>P. m. gambelii</i>	17	9	232	85.4	Frequent crises and relapses	Subacute, always fatal
(albino)						
<i>P. m. gambelii</i>	11	34	132	83.0	Frequent crises and relapses	Subacute, always fatal
(colored)						
<i>P. m. gambelii</i>	3	10	62	33.0	Frequent crises and relapses	Subacute, always fatal
(albino hairless)						
<i>P. m. gambelii</i>	13	35	207	109.7	Frequent crises and relapses	Subacute, always fatal
(colored hairless)						
<i>P. m. osgoodii</i>	4	45	116	78.5	Frequent crises and relapses	Subacute, always fatal
<i>P. m. rubidus</i>	11	34	82	49.0	Frequent crises and relapses	Subacute, always fatal
<i>P. m. rufinus</i>	25	18	224	102.8	Frequent crises and relapses	Subacute, always fatal
<i>P. m. sonorensis</i>	3	55	248†	129.7	Frequent crises and relapses	Subacute, always fatal
Hybrids F ₁						
<i>P. m. bairdii</i> Male × <i>P. m. gambelii</i> Female	43	25	347	83.6	Frequent crises and relapses	Subacute, always fatal
Hybrids F ₁						
<i>P. m. bairdii</i> Male × <i>P. m. rufinus</i> Female	23	16	381	116.4	Frequent crises and relapses	Subacute, always fatal

TABLE 1—Continued.

Species	No. animals inoculated with <i>Tr. brucei</i>	Fatality in days			Remarks	
		Min.	Max.	Aver.	Crises and relapses of trypanosomes	Type of infection
Hybrids F ₁						
<i>P. m. rufinus</i> Male × <i>P. m. gambelii</i> Female	9	96	381†	130.7	Long latent period	Subacute to chronic, always fatal
<i>P. l. leucopus</i>	2	36	50	43.0	Crises and relapses	Subacute, always fatal
<i>P. l. noveboracensis</i>	23	7	553†	222.0	Very long latent period with occ. relapses and crises	Chronic, 50 per cent recovery
<i>Mus musculus</i> (albino and colored)	128	3	10	6.2	No crisis	Acute, always fatal
<i>Mus musculus</i> (hairless)	12	2	7	5.0	No crisis	Acute, always fatal
<i>Mus bactrianus</i> (from China)	12	3	10	7.0	No crisis	Acute, always fatal
<i>Rattus rattus</i> (from Africa)	12	9	12	9.5	No crisis	Acute, always fatal
<i>Rattus norvegicus</i>	100	4	10	6.5	No crisis	Acute, always fatal
<i>Dipodomys merriami</i> . (Kangaroo rat)	1	7	7	7.0	No crisis	Acute, always fatal
<i>Eptesicus fuscus</i> (bats)	2	16	16	16.0	Crisis and relapse (?)	Acute to subacute, always fatal
Guinea pig	318	9	28	20.0	Crisis and relapse (occ. crises and relapses)	Acute subacute, always fatal
Cat	7	18	56	37.8	Crises and relapses	Acute subacute, always fatal
Rabbit	6	29	84	49.0	Crises and relapses	Acute subacute, always fatal

* All animals were inoculated heavily with *Tr. brucei* (0.25 cc. to 0.5 cc. suspension containing from 25 to 100 trypanosomes per microscopic field). Rabbits, cats and guinea pigs were inoculated subcutaneously, all other species were inoculated intraperitoneally.

† Signifies that certain number of animals are still alive. Therefore the maximum and the average fatality will be more than the figures indicate at the time of computing this table.

albino and hairless *Mus musculus* contracted acute infections and were all dead within a week.

The species *maniculatus*, then, exhibits pronounced resistance to nagana and on this basis can easily be distinguished from *P. c. californicus*, *P. c. insignis*, *P. e. eremicus*, *P. e. anthonyi*, *P. p. polionotus*, *Mus bactrianus*, and *Mus musculus*.

Hybrids (F_1) between *P. m. bairdii* (male) and *P. m. rufinus* (female), likewise between *P. m. bairdii* (male) and *P. m. gambelii* (female), exhibited approximately the same resistance to nagana as their parents. Since both of the parents had a high resistance to *Trypanosoma brucei*, these findings might have been expected; but it should be noted that (F_1) hybrids from the mating of *P. m. rufinus* (male) with *P. m. gambelii* (female) showed a more pronounced resistance to this organism. The latent periods were very long, and the average duration of the disease was greater than in any other form of *maniculatus*. (See table 1.)

Species in which nagana ran a chronic course from which the majority of animals apparently recovered.—*Peromyscus leucopus noveboracensis* was found to be extremely resistant to *Tr. brucei*, although occasionally infection took an acute to subacute course and terminated fatally. In a large percentage of this species, nagana ran a chronic course leading to apparent recovery.

The latent periods were very long, and in about 40 per cent of the individuals no relapses were demonstrable.

Within the body of *P. l. noveboracensis*, the parasite was gradually but markedly attenuated. This was manifested by the lengthening of the incubation period in susceptible animals. The actual period of recovery varied among different individuals, but most of the animals recovered in the course of several months, and a relative immunity was apparently established.

When relatively immune animals were inoculated with massive doses two, three, or even four times, at various intervals, the parasites failed to populate the blood. Although a small number of trypanosomes survived in the circulation of the animals for a short time, they were prevented from increasing in number by the protective mechanism of the host.

Seven mice (*P. l. noveboracensis*) which were inoculated September 1, 1932, and Nov. 21, 1932, are still alive and in good health 553 and 471 days later (March 7, 1934). These mice have been reinoculated several times with the same strain of *Tr. brucei*. In one of these animals, during the period of relative immunity, a slight infection was

detectable, 361 days after the initial inoculation. Although this late relapse strain was somewhat attenuated for susceptible animals, nevertheless it produced infections which terminated fatally in all of the animals inoculated with it. This suggests that the immunity established in *P. l. noveboracensis* is not absolute but relative.

Massive and frequent reinoculations of *P. l. noveboracensis* with *Tr. brucei* were usually accompanied by an increase in the virulence of the organisms in the blood (approaching its original pathogenicity for susceptible animals), but successive reinoculations did not increase the chances of the trypanosomes overpowering the host. Superinoculations, even at frequent intervals, failed to provoke a definite relapse. Following massive reinoculations daily blood examinations were almost always negative.

The number of parasites in the circulation of these reinoculated animals was so very small that inoculations into susceptible animals were necessary in establishing the presence of organisms. The trypanosomes were gradually attenuated by the protective mechanism of the host, until its blood was no longer infective to *Rattus norvegicus* and *Mus musculus*.

When the animal has recovered from reinfections, it is quite possible that all the trypanosomes have been destroyed, at least in the blood; if a few latent ones do exist they are so attenuated, that they can not produce any detectable infection in subinoculated test animals. These reinoculated *P. l. noveboracensis* are considered relatively immune to further reinoculation with *Tr. brucei*.

Discussion.

It is interesting to note that the intensity of infection and fatality varied so greatly in closely related species. The susceptibility of a given species for the same inoculum was relatively constant, though variations did occur. Such variations probably resulted from (a) the age factor; (b) the occurrence of intercurrent diseases (organic or infectious); (c) the care which the animals received (2); (d) pregnancy (12); (e) and above all the constitutional differences between individuals, even from the same parents (13).

It has been observed that mutations are usually injurious and detract from the vigor of the animals, while (F₁) hybrids between closely related species are often more vigorous than the parents. Thus hairless mutants of *Mus musculus* were slightly more susceptible to nagana than normal individuals, while hybrids between *P. m. rufinus* (male) and *P. m. gambelii* (female) were more resistant than either of their parents.

The mechanism of crises and relapses. Most authors (5, 6, 7, 8, 15, 16, 18) agree that a crisis seems to be associated with the appearance of antibodies in the blood. At this stage most of the parasites are destroyed, only a few remaining in the blood or possibly latent in the tissues. When the circulating antibodies have decreased, the parasites multiply and give rise to a relapse which may again stimulate the production of antibodies. These in turn cause the trypanosomes to disappear from the blood again. If the animals are unable to produce enough antibodies during the course of infection, the disease will end fatally. A few investigators, on the other hand, believe that crises and relapses depend specifically upon the developmental cycle of the trypanosomes, rather than upon the protective mechanism of the host.

Evidence is gradually accumulating, however, to indicate that crises are associated with the production of antibodies. Novy and Knapp (8) from work on relapsing fever spirochetes, believe that besides the germicidal action, an immunizing action also is present in the serum, and that the relative amount of these two at the close of an attack determines whether or not a relapse will occur. It is a state in which a working equilibrium is established between host and parasite, and like other equilibria it is subject to disturbance.

Crises and relapses of Tr. brucei in various subspecies of P. maniculatus and in P. l. noveboracensis. In these species it was noted that the latent period was relatively longer than the relapse period. During the latent periods and relapses, the number of trypanosomes in the blood varied from species to species and in different members of the same species. This irregularity was noted despite the fact that all of the animals were inoculated at the same time, with the same material and given the same care. Undoubtedly, the different individuals varied somewhat in their reaction to the parasite.

Occasionally when a second definite relapse occurred, it killed the animal, its capacity to develop antibodies seeming to have been lost or to have been present to such a small degree that the parasites were able to overpower the host. However, third, fourth, and occasionally fifth latent periods followed by relapses were also noted in certain subspecies of *maniculatus*.

Although the trypanosomes were not easily demonstrable microscopically in the blood of these animals during the latent period, susceptible animals inoculated with such blood became infected, except in the case of *P. l. noveboracensis*, in which animal inoculations were often negative. At times the disease appeared in test animals after a long incubation period. During latent periods in *maniculatus*, it re-

quired from 5 to 30 minutes of microscopic search in order to find a single parasite, and sometimes even such an effort was fruitless. It is quite possible that if a long systematic examination were made during the latent phase in this species, a few trypanosomes could be found. It appears that, except in about 50 per cent of *P. l. noveboracensis*, complete recovery is a rather rare occurrence.

Experimental data indicate that even in certain individuals of *P. l. noveboracensis*, prolonged and indefinite latency was associated with a few surviving trypanosomes. Thus on March 1, 1933, 100 days after the initial inoculation, *P. l. noveboracensis* no. 16 was etherized and almost its entire heart blood, 0.5 cc., was injected into a rat. The latter animal was observed for five months and failed to show symptoms of nagana. The heart, lungs, liver, spleen, kidneys, brain, muscles, and part of the bone marrow and glands of mouse no. 16 were ground into pulp in an equal volume of Tyrode solution and introduced into the peritoneal cavity of a rabbit by means of regular surgical technique. This rabbit later developed symptoms of nagana and trypanosomes were demonstrated in its blood on April 3, 1933. Two drops of ear blood from this animal were inoculated into a *Rattus norvegicus* which contracted the disease and died after 8 days. Fifty-three days after the original inoculation, this rabbit was found dead.

This particular experiment shows that at least a few trypanosomes were present in the tissues or organs of *noveboracensis* no. 16—an animal which had been pronounced relatively immune on the basis of negative microscopic examinations of its blood, as well as on the basis of the noninfectivity of its blood to rats.

The question now arises, why did the entire heart blood of this mouse, when inoculated into the rat, fail to produce infection? Were the trypanosomes present in the tissues, but not in the blood? Or had the trypanosomes become attenuated to such an extent that they were unable to produce infection in the rat, while they retained their pathogenicity for the rabbit?

Novy in his classical work on bird trypanosomes (10), after 13 consecutive hours of careful and systematic microscopic search on one stained blood smear, was able to find only a single trypanosome. Yet a few drops of this blood gave a rich culture. He also obtained cultures of *Tr. lewisi* from apparently immune rats (9).

Taliaferro's (15, 16) extensive and careful work on *Tr. lewisi* indicates that when a trypanocidal crisis is permanently effective it is because the few parasites which escape destruction cannot produce a relapse because they are prevented from reproducing.

It is therefore certain that during a latent period, trypanosomes do not entirely disappear from the general circulation. There are undoubtedly always a few remaining, which, under favorable conditions, may repopulate the blood and in time break down the existing immunity and terminate the life of the host.

It is difficult to generalize as to whether or not relapse strains differ in their pathogenicity from the original strains. There is some evidence that relapse strains of *Tr. brucei* from *P. maniculatus* are more virulent and relapse strains from *P. l. noveboracensis* are less virulent than the original strains, particularly for *Mus musculus* and *Rattus norvegicus*. The attenuated relapse strains sometimes reverted to their original pathogenicity after successive passages through a susceptible animal such as the rat.

After a third or fourth relapse, *Tr. brucei* usually killed the host. However, it appeared that in about fifty per cent of the individuals of *P. l. noveboracensis*, the active immunity developed by the host was sufficient to prevent further invasion of the blood by trypanosomes.

These recovered animals were reinfected with *Tr. brucei* by reinoculating them with massive and repeated doses of virulent nagana blood. This usually produced a second mild infection. Occasionally, the second infection ran an acute to subacute course and ended fatally. However, the majority of these mice recovered from reinfection and were found relatively immune to further reinoculation.

It is not yet clear whether relapses will occur in these apparently cured animals (*P. l. noveboracensis*). One of these mice (mouse 12) showed a mild relapse of trypanosomes 344 days after its initial inoculation. The blood of the animal at that time was fatal to *Mus musculus* and to the guinea pig. The former test animal died 17 days after inoculation, while the latter animal succumbed to the disease on the thirty-third day.

At present, March 7, 1934 (471 to 553 days after the initial inoculation) some animals belonging to this species are alive and in perfect health. Trypanosomes cannot be demonstrated in their blood, either microscopically or by animal inoculation.

It is known that periodic trypanolytic crises in various species of domestic mammals are only temporarily successful, because the few remaining trypanosomes become resistant and sooner or later repopulate the blood of the host, causing the death of the animal. Such a condition was noted in various subspecies of *P. maniculatus*. On the other hand, we must remember that certain wild mammals such as antelopes (so-called trypanosome reservoirs) apparently never die from

trypanosomiasis (1, 3, 4, 5, 18). It is also known that a small percentage of cows, sheep and goats appear to recover from natural or experimental nagana.

The present investigation indicates that the experimental *Tr. brucei* infection in *P. l. noveboracensis* is more or less analogous to nagana in a reservoir host. This species of American deer mouse is certainly a valuable experimental animal for the study of immunity in *Tr. brucei* infections and perhaps for other pathogenic trypanosomiasis. Also any subspecies of *maniculatus*, particularly the albino *P. m. gambelii*, would appear to be a particularly suitable animal for maintaining stock strains of pathogenic trypanosomes. Whether or not other species of "wild mice" not included in this work will react similarly to *Tr. brucei*, remains to be investigated.

Summary.

For the first time, several species, subspecies and hybrids of the American rodent genus *Peromyscus* were inoculated with *Tr. brucei* and the result recorded.

1. *P. c. californicus*, *P. c. insignis*, *P. e. eremicus*, *P. e. anthonyi* and *P. p. polionotus* contracted an acute infection, and all died within ten days.

2. *P. maniculatus* had a high resistance to the disease. The following subspecies of *maniculatus*, *P. m. artemisiae*, *P. m. bairdii*, *P. m. blandus*, *P. m. gambelii* (including albino, and hairless mutants), *P. m. osgoodii*, *P. m. rubidus*, and *P. m. sonorensis*, all contracted a subacute infection. Frequent crises and relapses were noted. The average duration of the disease was over 80 days, and the blood was invariably fatal to susceptible test animals.

3. F_1 hybrids between *P. m. bairdii* (male) and *P. m. rufinus* (female) behaved very much like their parents; F_1 hybrids from matings of *P. m. rufinus* (male) with *P. m. gambelii* (female) appeared more vigorous and manifested more resistance to the disease than either parent.

4. *P. l. noveboracensis* had very high resistance to nagana, the disease running a subacute to chronic course. Relapses were noted only in a few individuals. After several months many of these animals apparently recovered from the disease, and their blood was no longer infective to *Rattus norvegicus* and *Mus musculus*. Reinoculations produced a second mild infection, but the trypanosomes were unable to increase in the blood and after a few months the blood was again noninfective to *Rattus norvegicus* and *Mus musculus*. Several *Peromyscus leucopus noveboracensis* which have been reinoculated four

times with massive doses of *Tr. brucei* are still alive, 471 to 553 days after the original inoculation. These animals are considered relatively immune to further inoculations with *Tr. brucei*.

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